Helical Interactions in the HIV-1 gp41 Core Reveal Structural Basis for the Inhibitory Activity of gp41 Peptides

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Introduction: The envelope glycoprotein of HIV-1 consists of the surface subunit gp120 and the transmembrane subunit gp41. Binding of gp120 to target cell receptors induces a conformational change in gp41, which then mediates the fusion of viral and cellular membranes. The core structure of fusion-active gp41 is a six-helix bundle in which an N-terminal three-stranded coiled coil is surrounded by a sheath of antiparallel C-terminal helices. A conserved glutamine (Gln 652) buried in this helical interface replaced by leucine increases HIV-1 infectivity.

Methods and Materials: The Q652L variant was crystallized by the sitting drop vapor diffusion method at room temperature. To grow crystals, a 10 mg/ml HPLC-purified peptide stock was diluted 1:1 with a reservoir and allowed to equilibrate against the reservoir solution. Crystals of Q652L in space group *R*3 ($a = b = 52.38$ Å, $c = 60.48$ Å) were grown from 0.2 M ammonium sulfate, 0.1 M sodium acetate, pH 4.6, and 11% polyethylene glycol 4000 and transferred to a cryoprotected solution containing 15% (v/v) glycerol in the corresponding mother liquor.

Results: We investigate the role of the Gln 652 to Leu substitution on the conformation, stability, and biological activity of the N34(L6)C28 model of the gp41 ectodomain core. The 2.0 Å resolution crystal structure of the mutant molecule shows that the Leu 652 side chains make prominent contacts with hydrophobic grooves on the surface of the central coiled coil. The Gln 652 to Leu mutation leads to a marginal stabilization of the six-helix bundle by -0.8 kcal/mol, evaluated from thermal unfolding experiments. Strikingly, the mutant N34(L6)C28 peptide is a potent inhibitor of HIV-1 infection, with 10-fold greater activity than the wild-type molecule. This inhibitory potency can be traced to the corresponding C-terminal mutant peptide that likely has greater potential to interact with the coiled-coil trimer. Thus, the conserved interhelical packing interactions in the gp41 core offer a test-bed for the development of more potent analogs of gp41 peptide inhibitors.

Conclusions: Our results indicate that the interhelical interactions within the six-helix bundle structure of gp41 play a role in HIV-1 entry and its inhibition. We propose that the receptor-triggered conformational changes of the HIV-1 envelope glycoprotein are thermodynamically controlled, and that the process of membrane apposition and lipid bilayer fusion is driven by the currency of energy released from the formation of the fusion-active gp41 core. We suggest that the conformational state and membrane fusion activity of the gp120/gp41 complex are mechanistically coupled and thermodynamically linked.

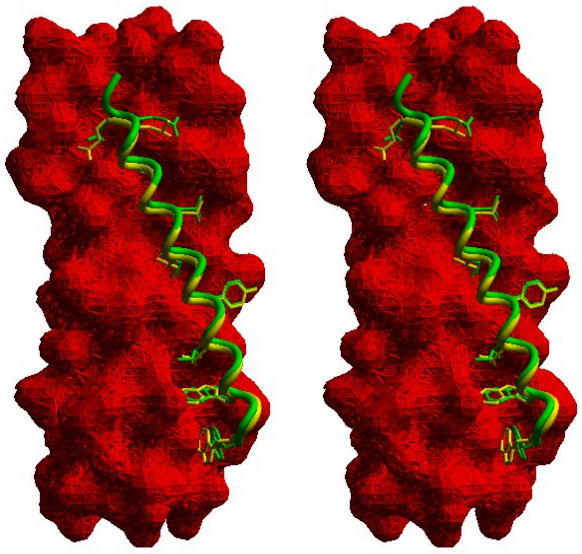

Figure 1. Stereoview of the interaction of the C28 helix with the conserved hydrophobic groove on the surface of the N34 coiled coil in the Q652L mutant gp41 core. The C28 helices of wild-type N34(L6)C28 (green) and Q652L (yellow), represented as ribbons, are shown against a surface representation of the central N34 coiled coil in wild-type N34(L6)C28.